AD-A180 015 MODULATION OF PHOSPHOLIPASE A2 ACTIVITY BY ACTIN AND MYOSIN(U) ARMY RESEARCH INST OF ENVIRONMENTAL MEDICINE NATICK MA D A DUBOSE ET AL 15 APR 87 1/1

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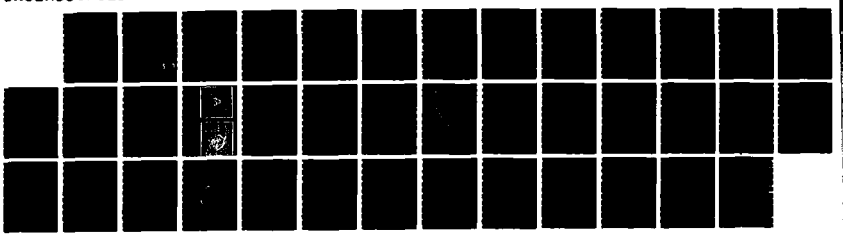

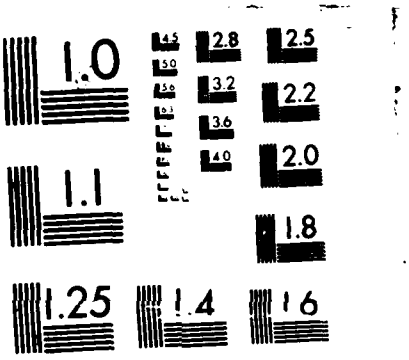

Modulation of Phospholipase A_2 Activity by Actin and Myosin

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Running title: Actin and myosin PLA_2 regulation

ABSTRACT

Endothelial cell prostacyclin (PGI_2) production is closely coupled with cell shape. Since shape change implies cytoskeletal modulations, this finding may be an important clue to implicate cytoskeletal constituents in a mechanism regulating eicosanoid metabolism. Endothelial cells with a diffuse F-actin distribution generate more PGI_2 than cells with many discrete F-actin stress fibers. Because actin can modulate enzyme activity, the myofibril protein effects on phospholipase A_2 (PLA_2), the rate limiting eicosanoid cascade enzyme, were studied. PLA_2 action on rat red blood cell ghost (RBC-G) membrane phospholipids resulted in ghost hemolysis. Enzyme activity was followed by measurement of the RBC-G bath supernatant absorbance at 418 nm. PLA_2 was exposed to actin (skeletal, smooth, or nonmuscle cell) at physiologically relevant concentrations (100 μM) for nonmuscle cells and then allowed to hemolyze the RBC-G. Comparisons of PLA_2 activity in the presence or absence of actin revealed that significantly ($p<0.05$) F-actin stimulated whereas G-actin suppressed enzyme activity. When myosin was introduced at a 10 or 100:1 F-actin to myosin ratio, the F-actin stimulatory effect was significantly ($p<0.05$) reduced. These findings suggest that the correlation of endothelial cell PGI_2 metabolic modulation to cell shape and actin distribution was perhaps due to changes in PLA_2 activity as a direct result of alterations in the polymerized state of actin and the degree of actin-myosin stress fiber formation.

INTRODUCTION

Phospholipase A_2 (PLA_2)[1] is the rate limiting eicosanoid cascade enzyme which initiates endothelial cell prostacyclin (PGI_2) metabolism (14). Other than a potential calcium regulatory role (25), little is known about the cellular mechanisms which control synthesis of this important arachidonic acid metabolite. However, cell shape is one factor which may impact eicosanoid metabolism. Bradykinin stimulates PGI_2 production by substrate-attached endothelial cells (10). In contrast, trypsin-detached endothelial cells in suspension are not stimulated by bradykinin (13). Moreover, transformed fibroblasts, whether detached by trypsin or with a rubberpoliceman, also lose their capacity to respond to bradykinin (10). Thus, the responses of substrate-attached and suspended cells are perhaps the result of cell shape differences and not due to the action of trypsin on membrane receptors.

Differences in cell shape might also explain the phenomenon in which PGI_2 synthesis by the small, rounded endothelial cells in contact-inhibited cultures is greater than that of large, flat cells in nonconfluent cultures (5). Cell shape is perhaps of more significance to this phenomenon than cell to cell contact, for in the absence of contact inhibition, small, rounded endothelial cells still generate significantly greater quantities of PGI_2 than large, flat cells (4). Cell shape change implies modulation of the cytoskeleton. As illustrated in Fig. 1, small, rounded endothelial cells have a diffuse F-actin distribution and appear to lack F-actin stress fibers, whereas large, flat cells have many stress fibers. A significant enhancement in PGI_2 synthesis is associated with a diffuse F-actin distribution (Fig. 1a) and such synthesis is reduced when complexes of F-actin, myosin, and other myofibril proteins (stress fibers; Fig. 1b) are present (4).

Actin is partly responsible for cellular shape and form. Changes in its distribution and polymerized state affect cell morphology. Actin binds a number of proteins (12) and modulates glycolytic enzyme activity (21-23). The control of glycolysis by the actin allosteric alteration of enzyme catalytic site is proposed (16). This suggests that the actin cytoskeleton is associated with mechanisms for the control of metabolism. Because cell shape (4,5,10,13) and F-actin distribution (4) correlate with PGI_2 production, a PLA_2 regulatory mechanism associated with actin may be operational in endothelial cells.

To determine the potential for a cytoskeletal-mediated regulatory mechanism, the effect of actin and complexes of F-actin with myosin on PLA_2 activity was determined. Evidence is presented which demonstrates that actin state of polymerization and actin-myosin complexes altered PLA_2 activity <u>in vitro</u>. This indicates that the correlation of endothelial cell PGI_2 synthesis to cell shape and actin distribution may be the result of metabolic control of the eicosanoid pathway by a cytoskeletal-directed mechanism.

MATERIALS and METHODS

Materials

Calcium chloride, ATP, potassium chloride, guanidine hydrochloride, rabbit muscle actin, chicken muscle actin, chicken gizzard actin, bovine muscle myosin, chicken gizzard myosin, bovine albumin, phalloidin, and PLA_2 derived from bee venom (PLA_2-B) or porcine pancreas (PLA_2-P) were obtained from Sigma Chemical Co., St. Louis, MO. Human platelet actin was procured from Calbiochem Biochemicals, San Diego, CA. Sodium chloride and sodium acetate were purchased from Mallinckrodt, Paris, KY. Fisher Scientific (Fairlawn, NJ) was the source for Tris and EDTA. Penobarbital sodium used to anesthetize rats came from Abbott Laboratories, North Chicago, IL.

The compositions of buffers and solutions were as follows: Isotonic saline: 0.144 M sodium chloride, 2.0 M calcium chloride, pH 7.5; Hemolysis buffer: 0.05 M sodium chloride, 1.0 mM EDTA; Resealing solution: 1.5 M sodium chloride, 1.0 mM EDTA; Actin polymerizing solution: 50.0 mM potassium chloride, 2.0 mM calcium chloride, 1.0 mM ATP; Actin depolymerizing solution: 1.5 M guanidine hydrochloride, 1.0 M sodium acetate, 1.0 mM ATP, 20.0 mM Tris, 6.0 mM calcium chloride, pH 7.5.

Preparation of Red Blood Cell Ghosts (RBC-G)

RBC-G were prepared as previously described (3,9,19,29). Blood was obtained from anesthetized (52 mg penobarbital sodium/kg; iv) laboratory rats. After centrifugation (2,500g), the supernatant and buffy coat were aspirated. The red blood cells were washed in isotonic saline. To each 5 ml pellet volume, 50 ml of hemolysis buffer was added and stirred at room temperature for 30 min. A sufficient volume of resealing solution was then added to restore isotonicity. This mixture was stirred slowly for 1 h. Following resealing, the ghosts were collected by centrifugation (15000g). Ghosts were gently resuspended in isotonic saline, in such a manner, as not to disturb the dark red button in the center of the pellet. The suspended ghosts were separated from the lysis-resistant red blood cells comprising this red button. Ghosts were washed in isotonic saline until the wash solution absorbance value (418 nm) remained constant. The RBC-G were then adjusted to a 20% hematocrit.

Preparation of F- and G-actin

Polymerizing solution was employed to generate F-actin (17). Actin (5 mg/ml: chicken skeletal muscle, chicken smooth muscle, or human platelet nonmuscle cell) was suspended in this solution for 2 h at 5° C. Polymerization increased solution viscosity. Actin (5 mg/ml) was suspended in depolymerizing solution to obtain G-actin (8). To

account for possible direct polymerizing or depolymerizing buffer effects, these solutions were used as diluents for PLA_2 incubated in the absence of myofibril protein.

Lysis of RBC-G by Phospholipase A_2

Lysis of the RBC-G followed the procedures as illustrated in Fig. 2. RBC-G (20% hematocrit) were diluted in isotonic saline (1:5.33) to a final volume of 9.0 ml. The mixture was slowly stirred at 37° C. When well suspended, four 250 μl samples were collected; centrifuged (15,000g) for 1 min; the supernatant diluted (1:10) in distilled water, and measured in a spectrophotometer at 418 nm to determine background absorbance before PLA_2 addition. PLA_2-B (88 units/ml) was incubated (30 min; 5° C) alone or in the presence of actin (100 μM; G- or F-actin from bovine or chicken skeletal muscle). PLA_2-P (300 units/ml) studies employed G- or F-actin derived from chicken skeletal muscle, chicken smooth muscle, or human platelet nonmuscle cell sources (100 μM). Some PLA_2-P studies used incubations with phalloidin (100 μM) or bovine albumin (100 μM). Other PLA_2-P studies involved incubations first with F-actin (chicken skeletal muscle; 100 μM) and then phalloidin (100 μM). PLA_2-P was also incubated with F-actin (chicken skeletal muscle or human platelet; 100 μM) and then myosin (bovine muscle or chicken gizzard; 10 or 1 μM). Following enzyme incubation, an aliquot (80.0 μl) was added to the RBC-G suspension (8 ml) and gently stirred at 37° C. At 5 min intervals over a 30 min test period, four 250 μl samples were collected and supernatant absorbance determined as described above. Each study of PLA_2 activity was repeated in triplicate.

From the 12 samplings (4 samples per 5 min time period X 3 replications), a mean absorbance value was calculated and a third degree regression curve generated which described PLA_2 activity with time. PLA_2 activity curves were studied by analysis of

variance and Tukey computation (30) to determine significant ($p<0.05$) differences in enzyme activity following exposure to myofibril or nonmyofibril proteins.

RESULTS

Lysis of RBC-G by Phospholipase

Fig. 3 demonstrates the reproducibility of PLA_2 activity curves under identical experimental conditions. PLA_2 action on the RBC-G membranes resulted in phospholipid hydrolysis and ghost lysis. Hemoglobin was released to the RBC-G bath and increased bath solution supernatant absorbance. By measurement of absorbance, the degree of ghost lysis and PLA_2 activity could be determined. No significant ($p<0.05$) differences were noted in PLA_2 activity when experimental conditions were the same. Results were similar using PLA_2-B or PLA_2-P.

Effects of Actin on the Activity of PLA_2 Derived from Bee Venom

As illustrated in Fig. 4, prior incubation of PLA_2-B with actin (100 μM) altered the activity of this enzyme. Polymerized chicken muscle actin significantly ($p<0.05$) stimulated PLA_2-B activity (Fig. 4a). Incubation in the presence of monomeric actin had the opposite effect, for PLA_2-B was significantly ($p<0.05$) inhibited (Fig. 4b). Similar results were recorded when rabbit muscle actin was employed (data not shown).

Effects of Actin and Myosin on the Activity of PLA_2 Derived from Porcine Pancreas

The effects of prior incubation with actin (100 μM) on PLA_2-P were similar to those noted for PLA_2-B (Fig. 5). For skeletal muscle (chicken muscle; Fig. 5a,b),

smooth muscle (chicken gizzard; Fig. 5c,d), or nonmuscle cell (human platelet; Fig. 5e,f) actin, F-actin (100 μM) significantly ($p<0.05$) stimulated, while G-actin (100 μM) significantly ($p<0.05$) inhibited PLA_2-P activity. As illustrated in Figures 5c and 5e, prior PLA_2-P incubation with smooth muscle or nonmuscle cell F-actin could be associated with an initial suppression in enzyme activity over the first 10 min in the RBC-G bath. Significant stimulation occurred only after this time period had elapsed.

While prior incubation with albumin (100 μM) significantly ($p<0.05$) inhibited PLA_2-P activity (Fig. 6a), phalloidin (100 μM) had no effect (Fig. 6b). However, when PLA_2-P was first incubated in F-actin (chicken skeletal muscle) and then phalloidin, the stimulatory effect of polymerized actin was significantly ($p<0.05$) improved (Fig. 6c). Furthermore, incubation with F-actin (100 μM) followed by myosin (10 or 1 μM) exposure significantly ($p<0.05$) reduced the stimulation of PLA_2-P seen after incubation with F-actin alone (Fig. 7). Results were similar using chicken skeletal muscle actin with bovine muscle myosin or human platelet actin with chicken gizzard myosin. Incubation of PLA_2-P with myosin (10 μM) in the absence of F-actin had no independent inhibitory PLA_2-P effects (data not shown).

DISCUSSION

PLA_2 activity was followed by the determination of hemoglobin release, as a result of RBC-G lysis. Under identical conditions, activity curves were replicated with no significant differences (Fig. 3). Therefore, changes in enzyme activity within experiments were not the result of variability inherent to this approach. Moreover, this model was appropriate, for the use of an intact phospholipid membrane as the substrate approximated the in vivo conditions under which this enzyme functions.

While incubation with phalloidin, in the absence of other protein, had no effect (Fig. 6b), albumin inhibited PLA_2 (Fig. 6a). The lack of a phalloidin effect demonstrated that not all proteins at a concentration of 100 μM altered PLA_2 activity. Albumin binds many proteins and serves as a transport vehicle for materials within the vascular fluids (7). Albumin's PLA_2 inhibitory effect indicated that it perhaps functions to suppress deleterious enzymes that gain access to the blood.

PLA_2 activity was also altered by the state of actin polymerization. Fibrous actin stimulated, while the monomeric form reduced PLA_2 activity (Figs. 4 and 5). Moreover, the F-actin stimulatory effect was reduced by myosin (Fig. 7). Though any direct connection to actual cellular events must be made with utmost caution, these <u>in vitro</u> findings suggest a potential for PLA_2 regulation by a cytoskeletal-directed mechanism.

Traditional methods were used to promote actin polymerization (17). When incubated with enzyme, actin (5 mg/ml) exceeded the critical concentration required for <u>in vitro</u> polymerization (12). Because of actin dilution (0.05 mg/ml), the RBC-G bath conditions were not as favorable for fibrous actin. However, the PLA_2/F-actin interaction was maintained for a sufficient time period, because activity was enhanced by prior incubation under conditions which supported fibrous actin, but not G-actin (Figs. 4 and 5). Differences in PLA_2 activity were not due to the polymerizing and depolymerizing buffers, because these solutions were used as diluents when PLA_2 was incubated in the absence of actin. The further enhancement of enzyme activity by phalloidin (Fig. 6c) was additional evidence that the F-actin state was essential to PLA_2 stimulation. Phalloidin binds to F-actin in a 1:1 ratio (26) and lowers the critical concentration for polymerization (2,6). Furthermore, it stabilizes F-actin by preventing monomer dissociation (2). Therefore, fibrous actin stabilization perhaps explains the further enhancement of PLA_2 activity by F-actin with phalloidin. This augmentative effect of phalloidin (Fig. 6c) also indicated that depolymerization occurred in the RBC-G bath.

As illustrated in Figs. 5c and 5e, F-actin suppressed PLA_2 activity during the first 10 min in the RBC-G bath. This might be explained by an initial inhibition by a fiber length that impeded enzyme-substrate interactions which was overcome by actin depolymerization to a more optimal fiber size. The phalloidin effect (Fig. 6c) was evidence for depolymerization in the RBC-G bath. Thus, though F-actin stimulated PLA_2, fiber length may be another modulating factor. Because such an effect was not consistently noted, initial RBC-G bath fiber length was perhaps an uncontrolled variable.

Actin and myosin are major stress fiber constituents. Myosin had no independent inhibitory effects (data not shown), however, it reduced the F-actin stimulation of PLA_2 (Fig. 7) which suggests that stress fiber formation may also affect enzyme activity. Tropomyosin and α-actinin (10 μM) stress fiber proteins did not alter the PLA_2 stimulatory effect of F-actin (data not shown). Thus, if myofibril protein complexes (stress fibers) alter PLA_2 activity, it is perhaps due to the presence of myosin.

The mechanism by which cytoskeletal proteins altered PLA_2 activity was not known. However, the model for glycolytic enzyme modulation by cytoskeletal constituents may be germane (16). In this model, enzyme binding to cytoskeletal structures alters the catalytic site to change metabolic rate. If such a model is applicable to our findings, then the PLA_2/G-actin interaction may have modified the catalytic site to reduce enzyme activity. However, F-actin must have further changed enzyme configuration to permit more efficient membrane phospholipid hydrolysis. If PLA_2 inhibition by G-actin were merely due to the effect of a single actin molecule obstructing enzyme access to its substrate, then further inhibition would be expected when the enzyme was associated with actin polymers. Since this was not the case (Figs. 4 and 5), the actin effect on PLA_2 was perhaps the result of enzyme catalytic site modulation.

A reduction in the F-actin stimulation of PLA_2 by myosin may be the result of any of the aforementioned mechanisms. Myosin binding to actin could further modulate enzyme configuration, directly obstruct enzyme active site, or affect fiber length such that substrate and enzyme could not easily associate. In addition, myosin binding might result in a dissociation of PLA_2 from F-actin.

Such mechanisms may be relevant to various cell types, for all actin isoforms altered PLA_2 activity (Fig. 5). Its potential relevance to endothelial cells was further enhanced by the use of actin (100 μM) and myosin (1 μM) concentrations reported for nonmuscle cells (18). Furthermore, a nonmuscle cell ratio of actin (skeletal muscle or nonmuscle cell) to myosin (skeletal or smooth muscle cell) of a 100:1 effectively suppressed the PLA_2 stimulation by F-actin (Fig. 7). Because several endothelial cell PLA_2 types might function to initiate the eicosanoid cascade (14), the finding that diverse PLA_2 sources (bee venom and porcine pancreas) were similarly affected by actin was important, for it suggested that this may be a common PLA_2 attribute. Also, eicosanoid enzymes are regarded to be membrane bound, however, PLA_2 may be bound or soluble in the cytosol (1). Moreover, an enzyme's existence in a particulate or soluble phase is a variable dependent on the cell metabolic status (11,15,22,28). Finally, enzyme-membrane associations do not necessarily exclude these potential actin-myosin modulatory effects. Therefore, these PLA_2 findings may be pertinent to endothelial cell PLA_2 activity and explain the correlation of PGI_2 synthesis to cell shape and actin distribution.

A diffuse F-actin distribution in small, rounded, substrate-attached endothelial cells (Fig. 1a) supports greater PGI_2 production (4), because PLA_2 may be maximally stimulated by F-actin free of other myofibril proteins (Fig. 5a,c,e). In large, flat, substrate-attached cells (Fig. 1b), actin and myosin complexed in stress fibers might reduce enzyme activity (Fig. 7) and PGI_2 production (4). Because of the correlation

between stress fiber formation and cell adhesion (4,27; Fig. 1), in the total absence of adhesion, G-actin may become dominant within suspended cells to suppress PLA_2 activity (Figs. 4b and 5b,d,f) and PGI_2 synthesis (10,13). Furthermore, suspended endothelial cells are stimulated to undergo significant calcium influx (20). However, suspended cells do not generate PGI_2 (10,13) and endothelial cell PLA_2 may be calcium independent (14). Calcium does not appear to offer an adequate explanation for PLA_2 regulation and calcium PLA_2 stimulatory actions (25) could be the result of calcium effects on endothelial cell cytoskeletal structure. Therefore, the correlation of PGI_2 production with endothelial cell shape and actin distribution, coupled with this PLA_2 study supports a cytoskeletal-mediated mechanism for eicosanoid metabolic regulation.

Since PGI_2 markedly promotes endothelial cell stress fiber formation (24), a cytoskeletal-mediated mechanism was further supported. As illustrated (Fig. 8), factors which cause an actin-myosin dissociation might support PLA_2 activation. The resulting PGI_2 would mediate the return of actin-myosin complexes. Such complexes might reduce PLA_2 activity to suppress further PGI_2 synthesis. In conclusion, because PGI_2 augments stress fiber formation and the cytoskeletal proteins actin and myosin could potentially modulate PLA_2 activity, PGI_2 in concert with the cytoskeleton may act in a signalling loop in the control of endothelial cell eicosanoid metabolism.

FIGURE LEGENDS

Figure 1. The effects of substrate adhesive capacity on bovine aortic endothelial (BAE) cell shape, F-actin distribution, and actin stress fiber formation. BAE cells were seeded on bacteriological plastic or tissue culture plastic. After 24 h, cells were fixed (3.7% formalin; 30 min), treated with acetone (80%; 10 min), and exposed to rhodamine phalloidin (0.165 μM; 10 min; Junction City, OR). A Zeiss microscope equipped with the appropriate filters for the excitation of rhodamine was used to observe the cell fluorescence. Photographs of the fluorescence were taken using Tri-X Pan film (Kodac; 400 ASA). BAE cells on a substrate of reduced adhesive capacity (bacteriological plastic; Fig 1a) were small and rounded with a diffuse F-actin distribution which appeared to lack stress fibers. Such cells on an adhesive substrate (tissue culture plastic; Fig. 1b) were large and flat with many discrete actin stress fibers.

Figure 2. Phospholipase A_2 (PLA_2) was incubated (30 min; 5° C) in the presence or absence of myofibril protein prior to exposure to the red blood cell (RBC) ghost bath. Enzyme activity was followed by measurement of sample supernatant absorbance at 418 nm.

Figure 3. Illustration of phospholipase A_2 (PLA_2) activity curves replicated under identical experimental conditions. Similar PLA_2 activity curves were obtained using PLA_2 derived from bee venom or porcine pancreas

Figure 4. Effects of prior incubation with F-actin (A; 100 μM) or G-actin (B; 100 μM) derived from chicken skeletal muscle (sk) on bee venom phospholipase A_2 (PLA_2-B) activity.

Figure 5. Effects of prior incubation with F-actin (A,C, and E; 100 μM) or G-actin (B, D, and F; 100 μM) from chicken skeletal muscle (sk), chicken smooth muscle (sm), or human platelet nonmuscle (nm) cell sources on porcine pancreatic phospholipase A_2 (PLA_2-P) activity.

Figure 6. Effects of prior incubation with albumin (A; 100 μM), phalloidin (B; 100 μM), or chicken skeletal muscle (sk) F-actin plus phalloidin (C; 100 μM) on porcine pancreatic phospholipase A_2 (PLA_2-P) activity.

Figure 7. Effects of prior incubation with F-actin (100 μM) or F-actin (100 μM) plus myosin (10 or 1 μM) on porcine pancreatic phospholipase A_2 (PLA_2-P) activity. Similar PLA_2 activity curves were obtained using chicken skeletal muscle actin and such actin (skeletal muscle cell) in combination with bovine muscle myosin or human platelet actin and such actin (nonmuscle cell) in combination with chicken gizzard myosin.

Figure 8. Illustration of a potential cytoskeletal-prostacyclin (PGI_2) signalling loop which perhaps functions as an eicosanoid metabolic regulatory mechanism. Factors which cause a dissociation of F-actin from other myofibril proteins (i.e., myosin) may maximally stimulate the rate limiting enzyme of the eicosanoid cascade, phospholipase A_2 (PLA_2). Such stimulation would induce enhanced arachidonic acid release from cell membranes and elevate PGI_2 synthesis. The binding of prostacyclin to its punitive membrane receptor would initiate the formation of complexes of F-actin, myosin, and other myofibril proteins (stress fibers). The association of PLA_2 with such complexes might suppress PLA_2 activity and reduce PGI_2 metabolism.

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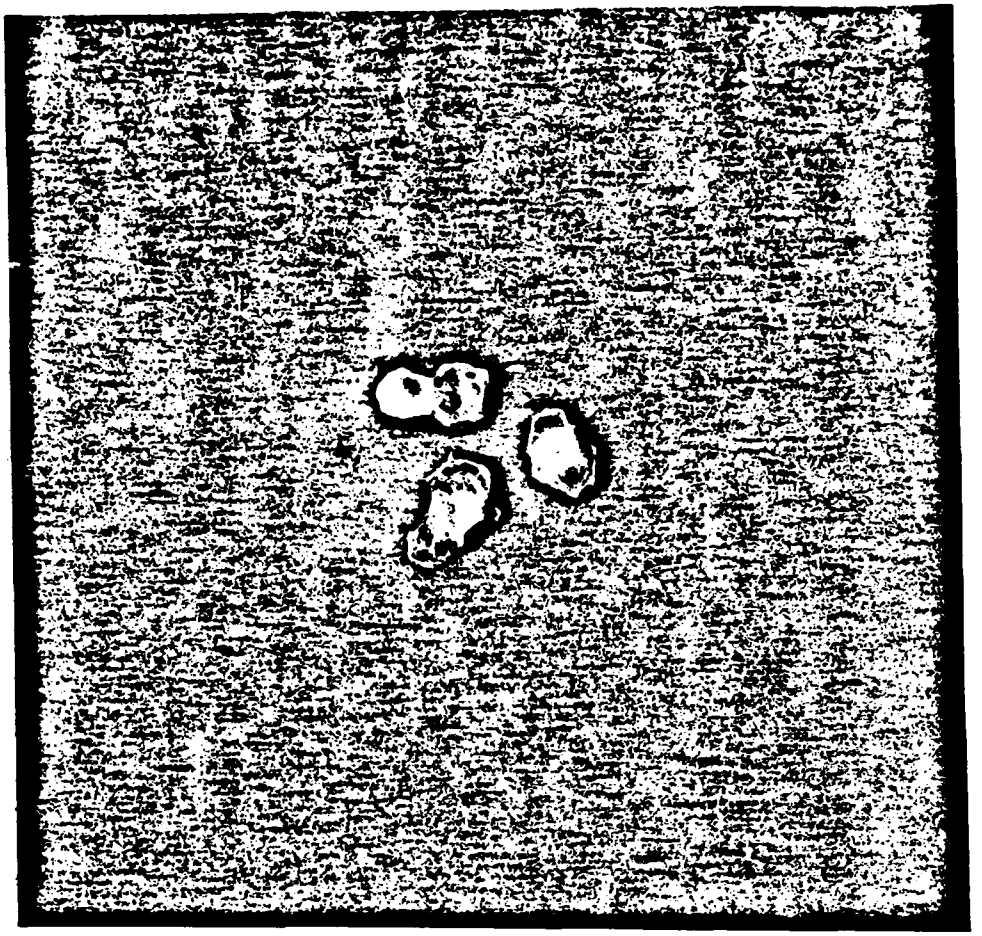
1A.



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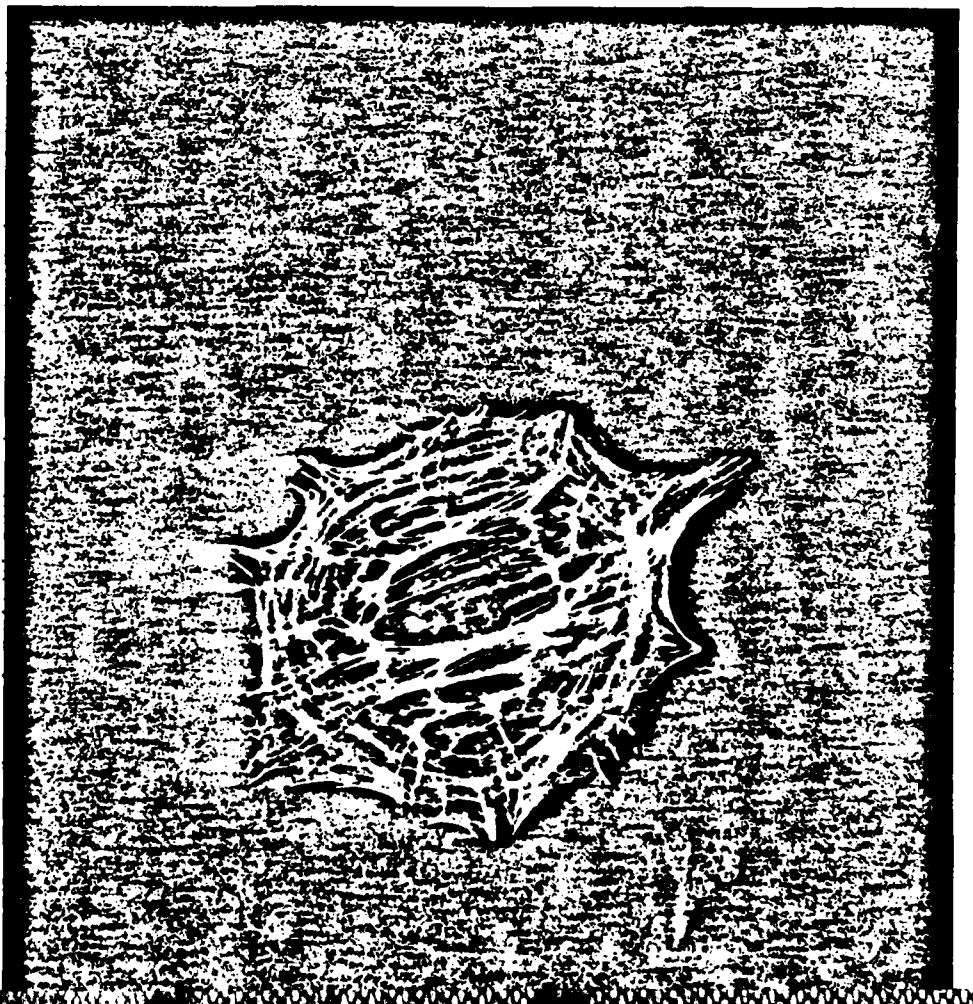
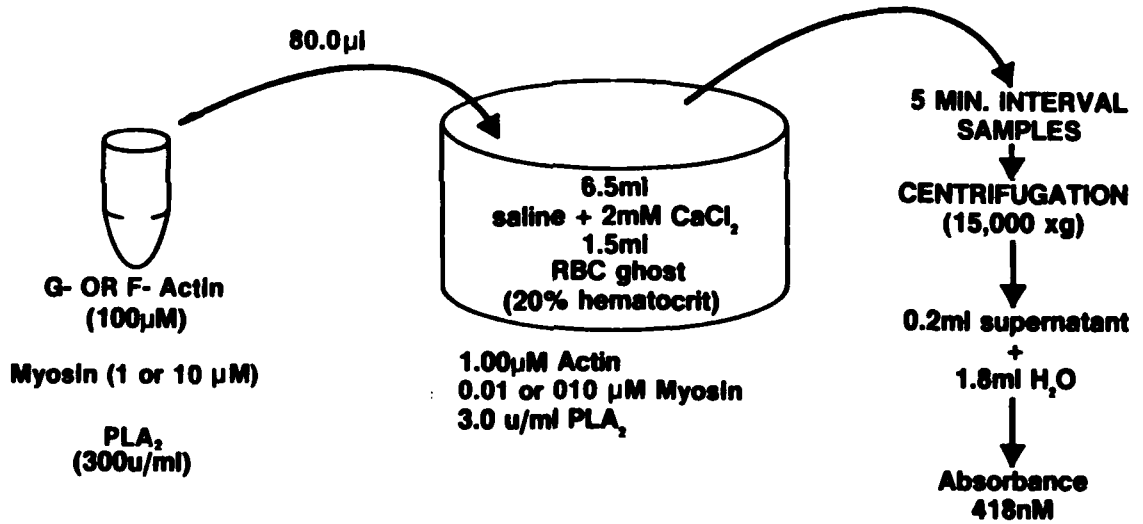


Figure 2. Phospholipase A_2 (PLA_2) was incubated (30 min; 5° C) in the presence or absence of myofibril protein prior to exposure to the red blood cell (RBC) ghost bath. Enzyme activity was followed by measurement of sample supernatant absorbance at 418 nm.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G- OR F- Actin
(100µM)
Myosin (1 or 10 µM)
PLA2
(300u/ml)
6.5ml
saline + 2mM CaCl2
1.5ml
RBC ghost
(20% hematocrit)
1.00µM Actin
0.01 or 010 µM Myosin
3.0 u/ml PLA2
5 MIN. INTERVAL
SAMPLES
CENTRIFUGATION
(15,000 xg)
0.2ml supernatant
+
1.8ml H2O
Absorbance
418nM

Figure 3. Illustration of phospholipase A_2 (PLA_2) activity curves replicated under identical experimental conditions. Similar PLA_2 activity curves were obtained using PLA_2 derived from bee venom or porcine pancreas

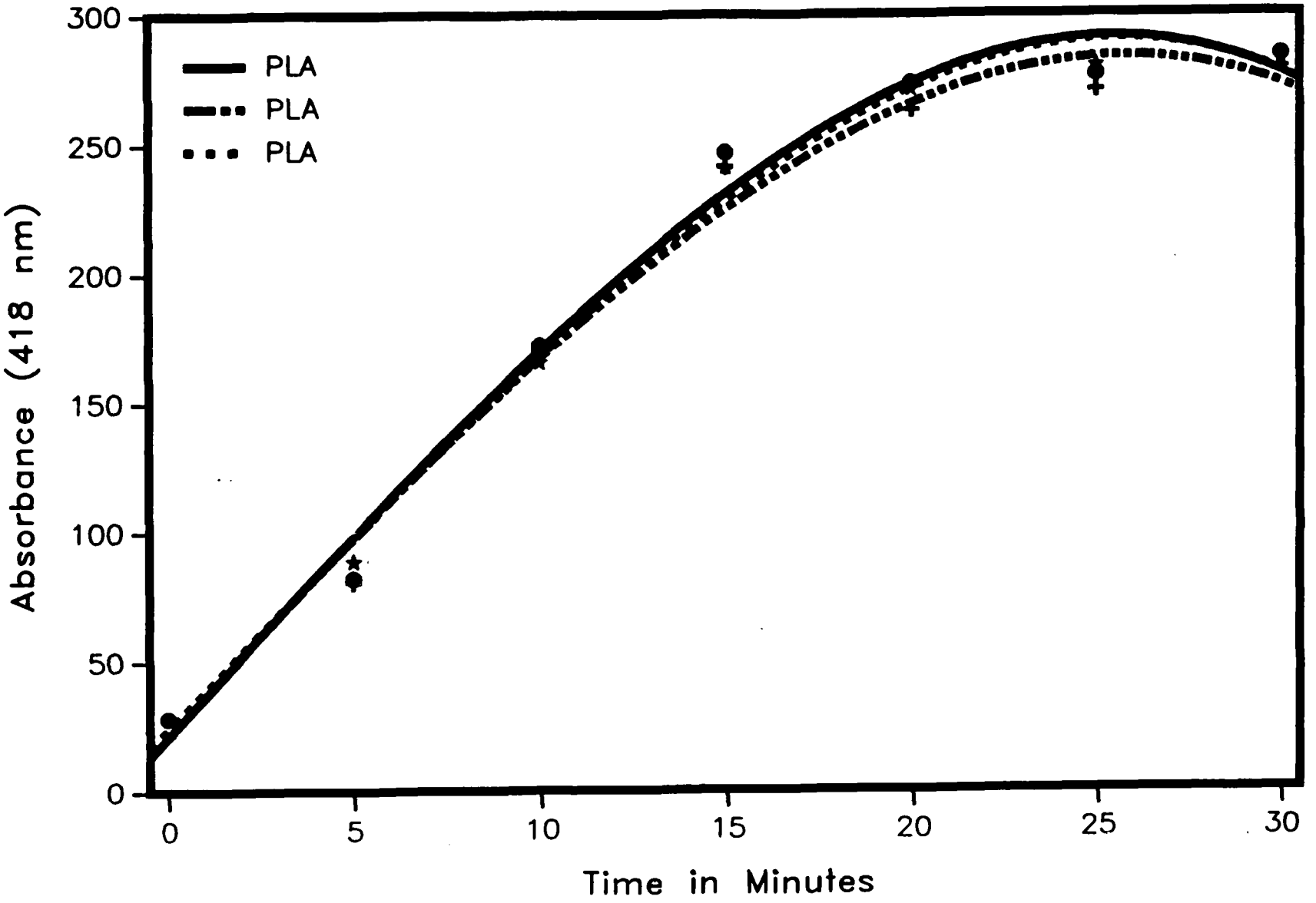
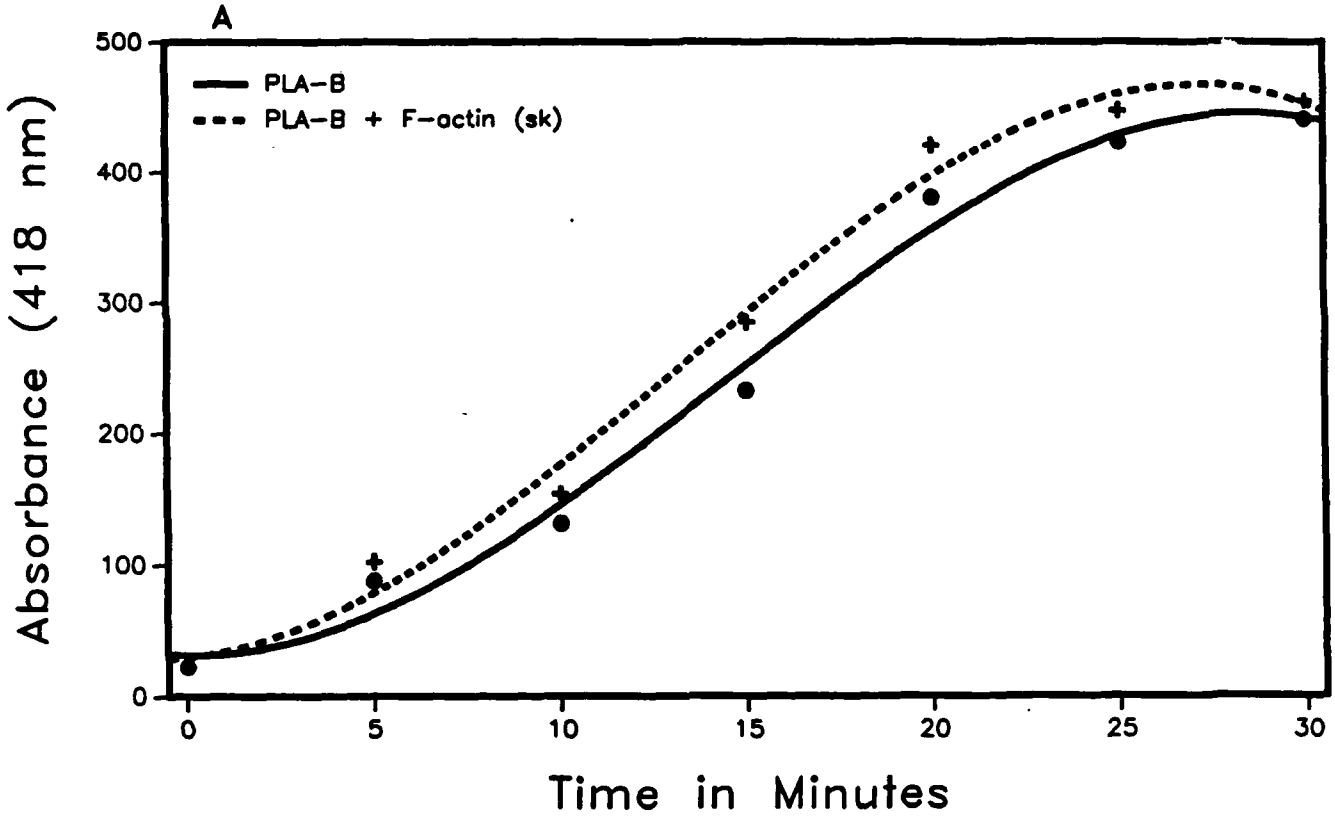

PLA
PLA
PLA
Absorbance (418 nm)
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Time in Minutes

Figure 4. Effects of prior incubation with F-actin (A: 100 μM) or G-actin (B: 100 μM) derived from chicken skeletal muscle (sk) on bee venom phospholipase A_2 (PLA_2-B) activity.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PLA–B
PLA–B + F–actin (sk)
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Time in Minutes

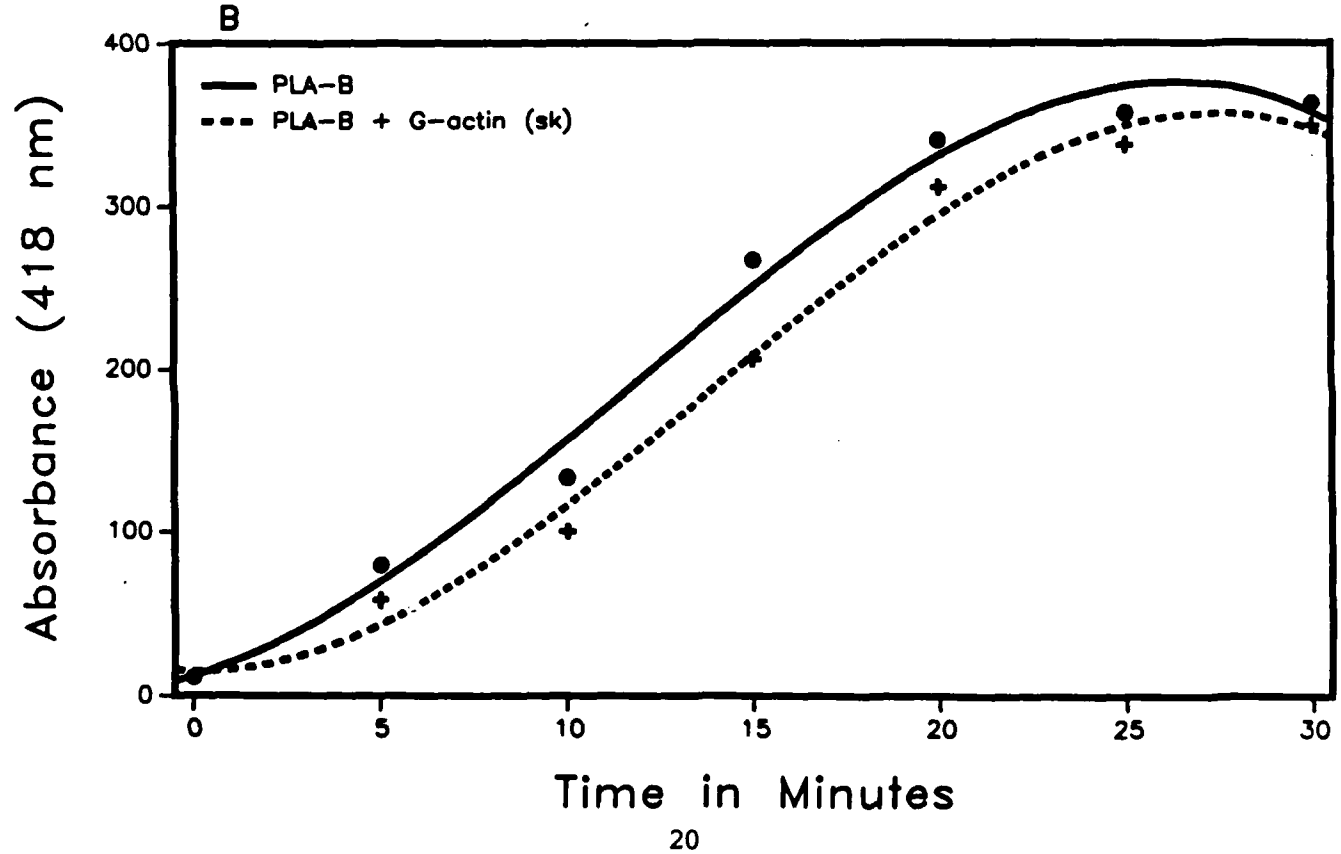

B
PLA–B
PLA–B + G–actin (sk)
Absorbance (418 nm)
400
300
200
100
0
0
5
10
15
20
25
30
Time in Minutes

Figure 5. Effects of prior incubation with F-actin (A.C. and E; 100 μM) or G-actin (B. D. and F; 100 μM) from chicken skeletal muscle (sk), chicken smooth muscle (sm), or human platelet nonmuscle (nm) cell sources on porcine pancreatic phospholipase A_2 (PLA_2-P) activity.

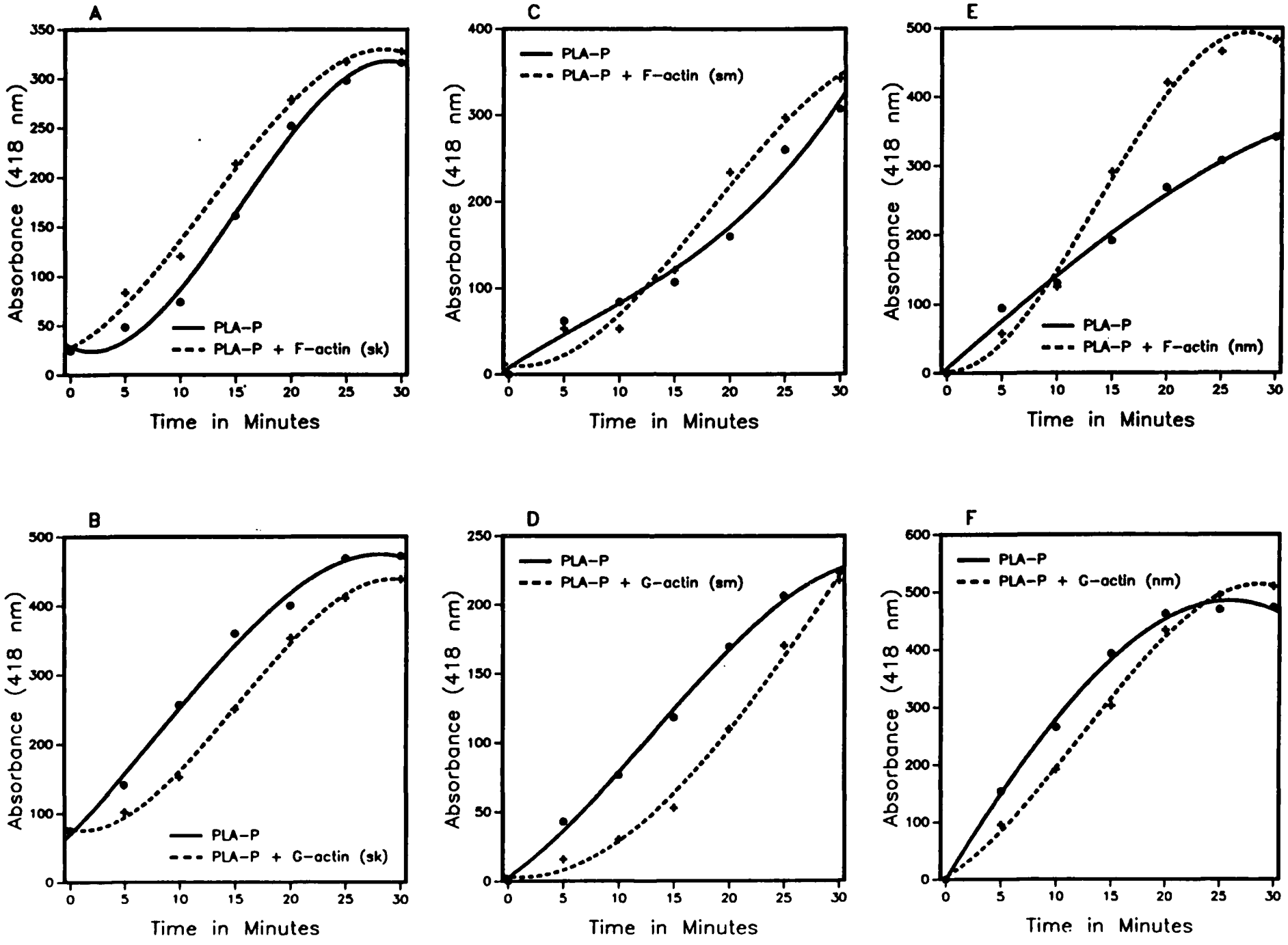
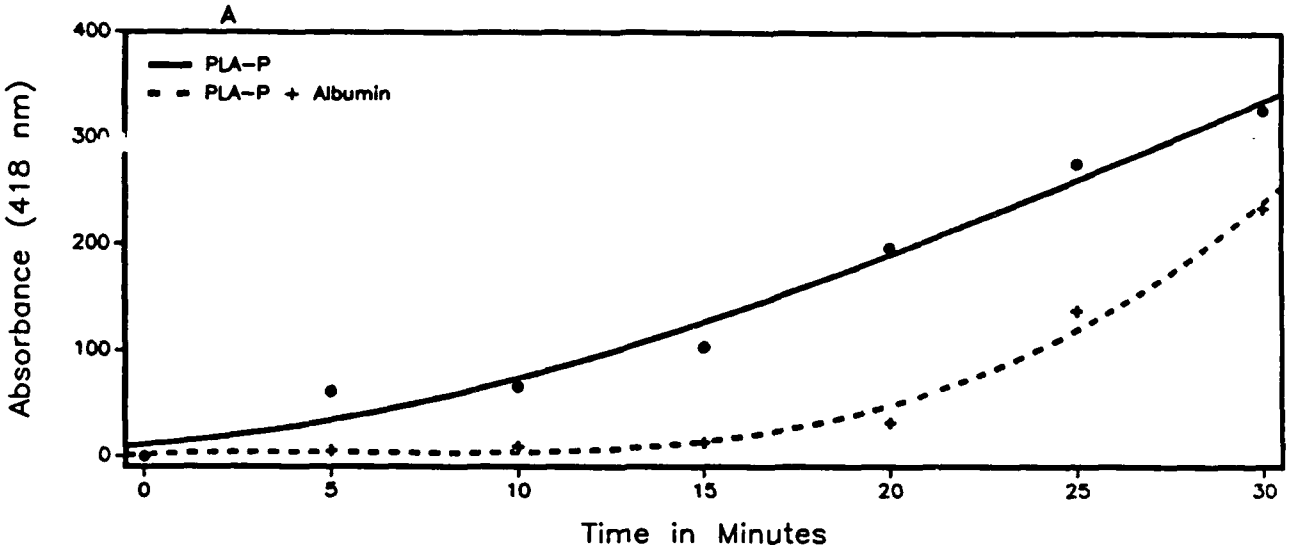

A
Absorbance (418 nm)
Time in Minutes
PLA-P
PLA-P + F-actin (sk)
C
PLA-P
PLA-P + F-actin (sm)
E
PLA-P
PLA-P + F-actin (nm)
B
PLA-P
PLA-P + G-actin (sk)
D
PLA-P
PLA-P + G-actin (sm)
F
PLA-P
PLA-P + G-actin (nm)

Figure 6. Effects of prior incubation with albumin (A: 100 μM), phalloidin (B: 100 μM), or chicken skeletal muscle (sk) F-actin plus phalloidin (C: 100 μM) on porcine pancreatic phospholipase A_2 (PLA_2-P) activity.


A
PLA-P
PLA-P + Albumin
Absorbance (418 nm)
400
300
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100
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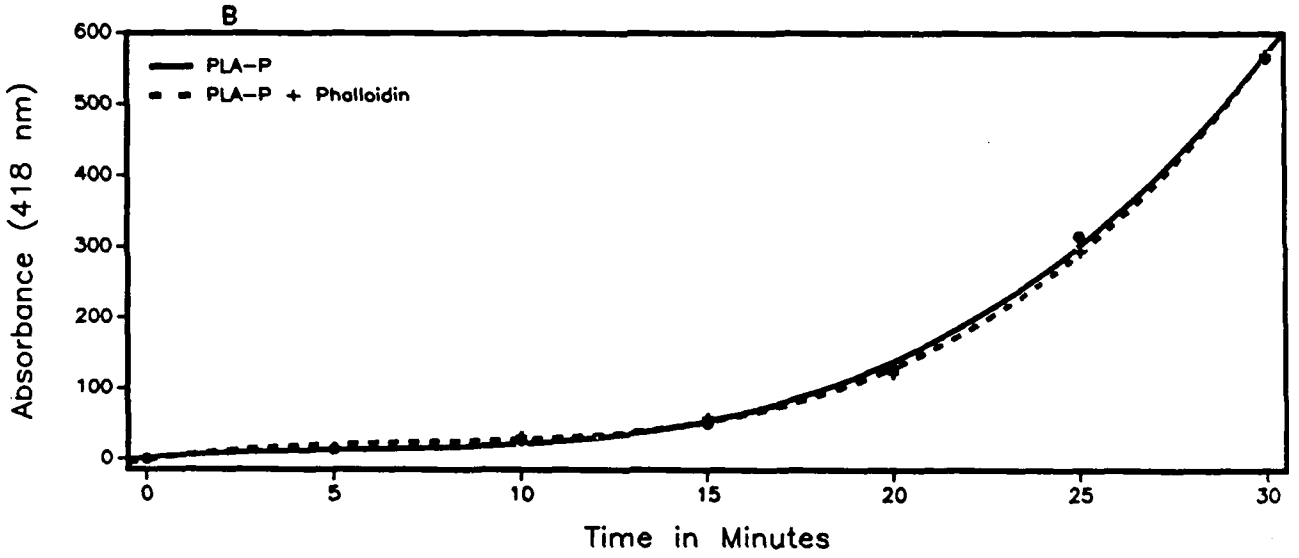
30
Time in Minutes


B
PLA-P
PLA-P + Phalloidin
Absorbance (418 nm)
600
500
400
300
200
100
0
0
5
10
15
20
25
30
Time in Minutes

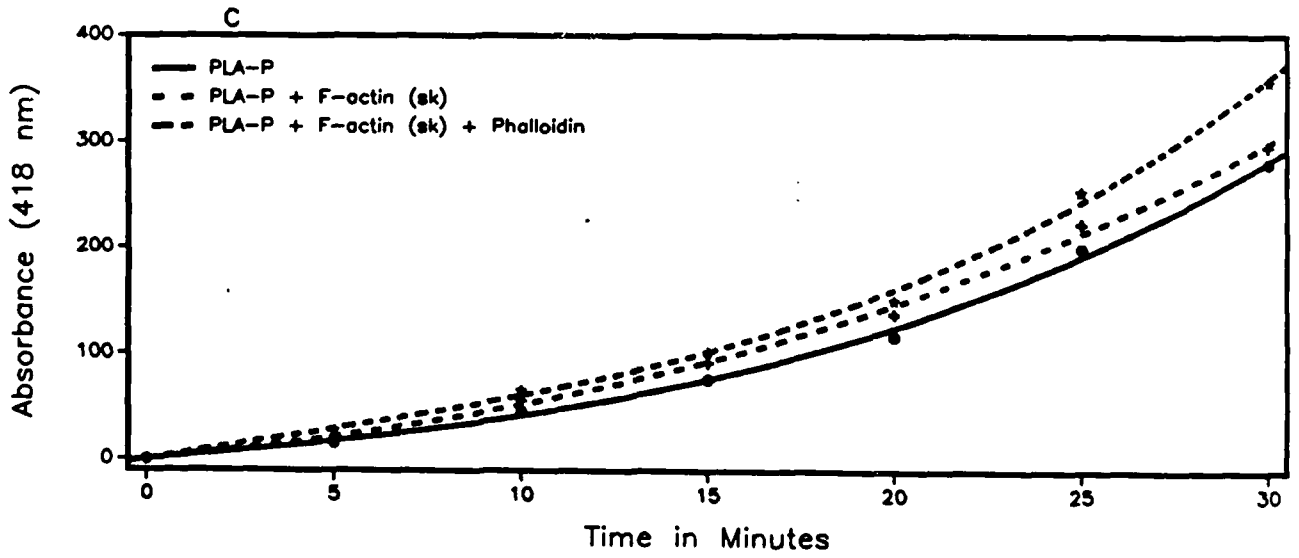

C
PLA-P
PLA-P + F-actin (sk)
PLA-P + F-actin (sk) + Phalloidin
Absorbance (418 nm)
400
300
200
100
0
0
5
10
15
20
25
30
Time in Minutes

Figure 7. Effects of prior incubation with F-actin (100 μM) or F-actin (100 μM) plus myosin (10 or 1 μM) on porcine pancreatic phospholipase A_2 (PLA_2-P) activity. Similar PLA_2 activity curves were obtained using chicken skeletal muscle actin and such actin (skeletal muscle cell) in combination with bovine muscle myosin or human platelet actin and such actin (nonmuscle cell) in combination with chicken gizzard myosin.

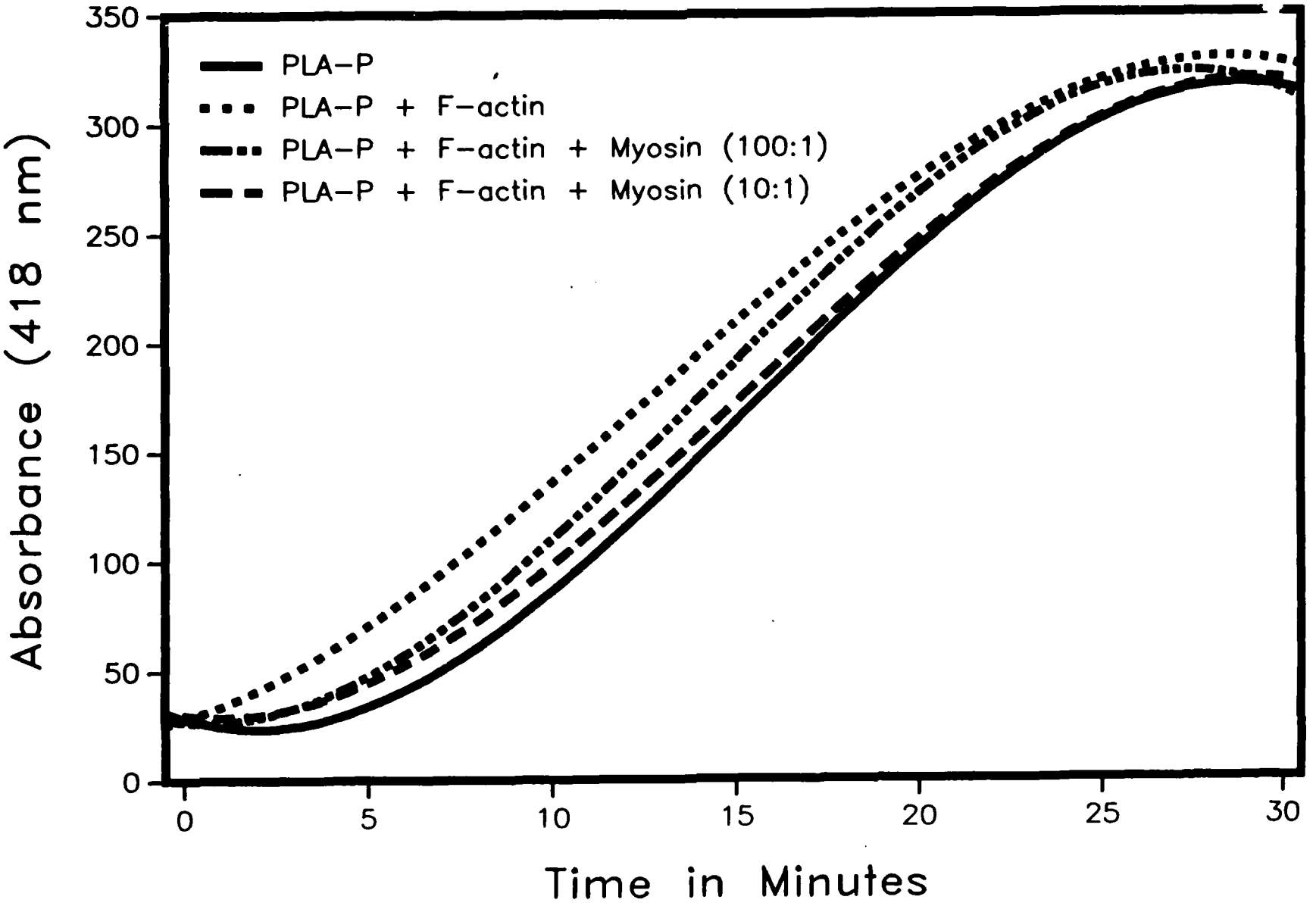
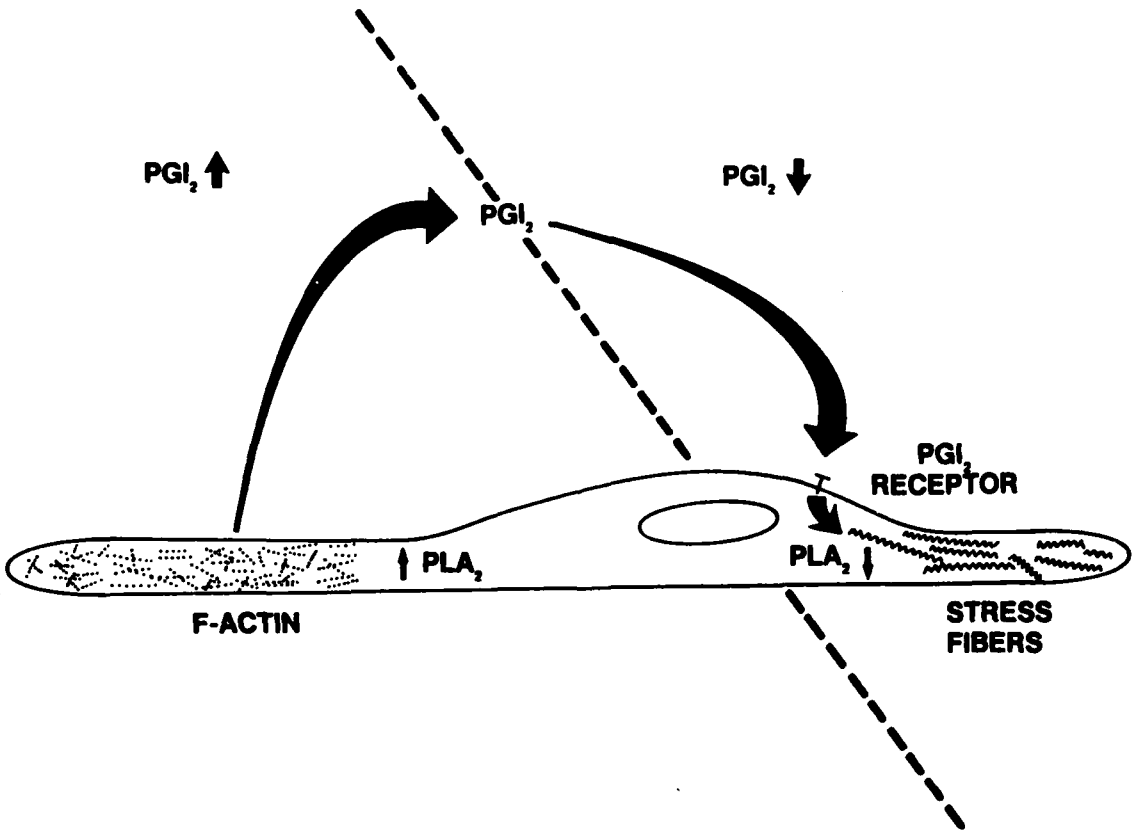

PLA–P
PLA–P + F–actin
PLA–P + F–actin + Myosin (100:1)
PLA–P + F–actin + Myosin (10:1)
Absorbance (418 nm)
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Time in Minutes

Figure 8. Illustration of a potential cytoskeletal-prostacyclin (PGI_2) signalling loop which perhaps functions as an eicosanoid metabolic regulatory mechanism. Factors which cause a dissociation of F-actin from other myofibril proteins (i.e., myosin) may maximally stimulate the rate limiting enzyme of the eicosanoid cascade, phospholipase A_2 (PLA_2). Such stimulation would induce enhanced arachidonic acid release from cell membranes and elevate PGI_2 synthesis. The binding of prostacyclin to its punitive membrane receptor would initiate the formation of complexes of F-actin, myosin, and other myofibril proteins (stress fibers). The association of PLA_2 with such complexes might suppress PLA_2 activity and reduce PGI_2 metabolism.


PGI2
PGI2
PGI2
PGI2
RECEPTOR
PLA2
PLA2
F-ACTIN
STRESS
FIBERS

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1. Abbreviations used: PLA_2, phospholipase A_2: PLA_2-B, phospholipase A_2 derived from bee venom; PLA_2-P, phospholipase A_2 derived from porcine pancreas; PGI_2, prostacyclin, RBC-G, red blood cell ghosts.

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DISCLAIMER

The investigator adhered to the "Guide for Laboratory Animal Facilities and Care as promulgated by the Committee on the Guide for Laoratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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Endothelial cell prostacyclin ~~(PGI)~~ production is closely coupled with cell shape. Since shape change implies cytoskeletal modulations, this finding may be an important clue to implicate cytoskeletal constituents in a mechanism regulating eicosanoid metabolism. Endothelial cells with a diffuse F-actin distribution generate more PGI than cells with many discrete F-actin stress fibers. Because actin can modulate enzyme activity, the myofibril protein effects on phopholipase A(PLA), the rate limiting eicosanoid cascade enzyme, were studied. F-actin stimulated wheras G-actin suppressed enzyme activity. Myosin in the presence of F-actin, the F-actin stimulatory effect was significantly reduced. These findings suggest that the correlation of endothelial cell PGI metabolic modulation to cell shape and actin distribution was perhaps due to changes in PLA activity as a direct result of alterations in the polymerized state of actin and the degree of actin-myosin stress fiber formation.

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